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Deriving the rate equations for product inhibition patterns in bisubstrate enzyme reactions

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Abstract

In this work, the full rate equations for 17 completely reversible bisubstrate enzyme kinetic mechanisms, with two substrates in the forward and two in the reverse direction, have been presented; among these are rapid equilibrium, steady-state, and mixed steady-state and rapid equilibrium mechanisms. From each rate equation eight product inhibition equations were derived, four for the forward and four for the reverse direction. All the corresponding product inhibition equations were derived in full; thus a total of $17 \times 8 = 136$ equations, were presented. From these equations a list of product inhibition patterns were constructed and presented in a tabular form, both for the primary plots (intercept effects) and the secondary plots (slope effects).

The purpose of this work is to help investigators in practical work, especially biologists working with enzymes, to choose quickly an appropriate product inhibition pattern for the identification of the kinetic mechanism. The practical application of above product inhibition analysis was illustrated with three examples of yeast alcohol dehydrogenase-catalyzed reactions.

Introduction

The majority of enzyme-catalyzed reactions in nature are bisubstrate reactions with two substrates and two products of reaction; monosubstrate reactions are rare, and trisubstrate reactions are far less numerous then bisubstrate (Enzyme nomenclature 1992; [11,16]). [6] and [2–4] have pioneered the derivation of rate equations for several major bisubstrate mechanisms, which was followed by other authors [16]. Several rate equations for major sequential bisubstrate mechanisms are presented *in extenso* in a textbook format by [17] and by [14] and in a review format in several volumes of Methods in Enzymology. However, the textbook and the review format published so far present only a fraction of all possible bisubstrate mechanisms that occur in nature.

The purpose of this article is: (a) To review the methods for the derivation of full rate equations for bisubstrate reactions and derive equations for most mechanisms that occur in nature. (b) Further, to derive all possible product inhibition equations in the double reciprocal form from the full rate equations. (c) Finally, the aim is to use the above product inhibition equations to construct the product inhibition patterns in a tabular form. In this way, the product inhibition tables can help the students in practical work with enzymes and help them to identify an appropriate kinetic mechanism in question. In order to learn how this is done in practical kinetics, students need to work some examples with real kinetic data.

Bisubstrate enzyme reactions

Bisubstrate enzyme reactions, with two substrates (A and B) and two products of the reaction (P and Q), can be classified into two broad categories: rapid

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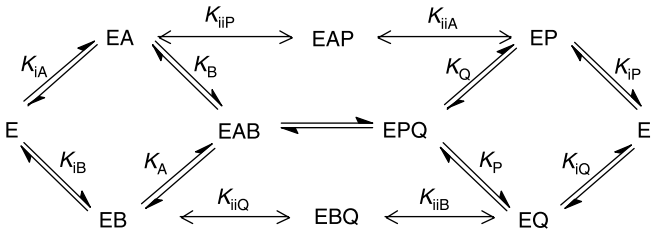
equilibrium Bi Bi and steady-state Bi Bi reactions. The derivation of rate equations for each category is very different and will be further treated in separate sections. In bisubstrate reactions, the designation of A, B, and P, Q are arbitrary; however, when dealing with NAD(P)-dependent dehydrogenases, it is customary to assign A to oxidized coenzyme and B to alcohol substrate in the forward direction, and P to carbonyl substrate and Q to reduced coenzyme in the reverse direction. This choice is not voluntary, but makes an important difference when one is dealing with tables of product inhibition patterns (Tables II – V).

Rapid equilibrium Bi Bi mechanisms

It is important to realize that in rapid equilibrium mechanisms all forms of enzyme are in a rapid thermodynamic equilibrium, which is established immediately after mixing the substrates with the enzyme. For rapid equilibrium systems, the rate equations can be obtained easily; no derivation is really necessary, and the rate equations can be written directly from an inspection of the equilibriums between the enzyme species [14]. The full rate equations for all rapid equilibrium Bi Bi mechanisms are easily derived from the general rate equation for the Rapid equilibrium random Bi Bi mechanism (*Scheme 1*) with EAP and EBQ complexes (Equation I).

Table I. Survey of rapid equilibrium Bi Bi mechanisms.

	Mechanism	Omission of respective enzyme form from *Scheme 1*, or respective denominator term from Equation (I)
1	RE Random Bi Bi without dead-end complexes	Delete EAP and EBQ
2	RE Random Bi Bi with EAP complex	Delete EBQ
3	RE Random Bi Bi with EBQ complex	Delete EAP
4	RE Random Bi Bi with EAP and EBQ complexes	No deletion
5	RE Random Bi Bi with EAP, EBQ, PE, and PEP complexes	Special case
6	RE Ordered Bi Bi without dead-end complexes	Delete EB, EP, EAP, and EBQ
7	RE Ordered Bi Bi with dead-end EAP complex	Delete EB, EP, and EBQ
8	RE Ordered Bi Bi with dead-end EBQ complex	Delete EB, EP, and EAP
9	Forward RE random, reverse RE ordered Bi Bi	Delete EP, EAP, and EBQ
10	Forward RE ordered, reverse RE random Bi Bi	Delete EB, EAP, and EBQ
11	RE ordered Bi Bi, with QE and QEQ complex	Special case



$$v_o = \frac{V_1\left(\frac{AB}{K_{iA}K_B}\right) - V_2\left(\frac{PQ}{K_P K_{iQ}}\right)}{1 + \frac{A}{K_{iA}} + \frac{B}{K_{iB}} + \frac{P}{K_{iP}} + \frac{Q}{K_{iQ}} + \frac{AB}{K_{iA}K_B} + \frac{PQ}{K_P K_{iQ}} + \frac{BQ}{K_{iB}K_{iiQ}} + \frac{AP}{K_{iA}K_{iiP}}} \quad \text{(I)}$$

Scheme 1.

In *Scheme 1* all kinetic constants represent true dissociation constants of the respective enzyme forms.

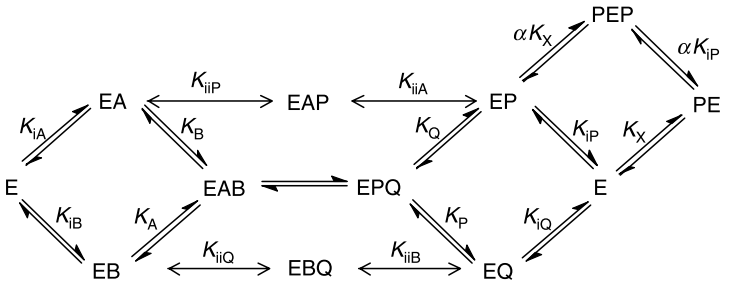
Summary of Rapid equilibrium Bi Bi mechanisms and rate equations

In this article, we shall describe 11 rapid equilibrium Bi Bi mechanisms which are listed in Table I. Each mechanism can be easily written down schematically simply by omitting from *Scheme* 1 the enzyme form indicated in Table I. Further, the full rate equation for each mechanism can be easily written down from Equation (I), simply by omitting from its denominator the terms indicated in Table I. The rapid equilibrium mechanisms always contain one denominator term for each enzyme form in the mechanism. The numerator term is always the same in all mechanisms.

Special cases in Rapid equilibrium Bi Bi mechanisms

There are two special cases in Rapid equilibrium Bi Bi mechanisms. The first is the Rapid equilibrium random Bi Bi with EAP, EBQ, PE, and PEP complexes.

Mechanism 5 (Scheme 2).



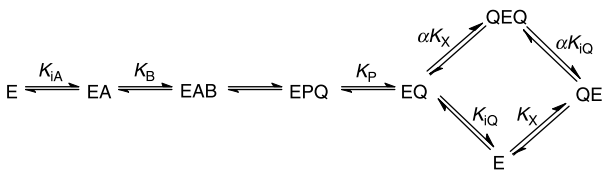
$$v_o = \frac{V_1\left(\frac{AB}{K_{iA}K_B}\right) - V_2\left(\frac{PQ}{K_P K_{iQ}}\right)}{1 + \frac{A}{K_{iA}} + \frac{B}{K_{iB}} + \frac{P}{K_{iP}} + \frac{Q}{K_{iQ}} + \frac{AB}{K_{iA}K_B} + \frac{PQ}{K_P K_{iQ}} + \frac{BQ}{K_{iB}K_{iQ}} + \frac{AP}{K_{iA}K_{iP}} + \frac{P}{K_X} + \frac{P^2}{K_X K_{iP}}} \quad \text{(II)}$$

Scheme 2.

In this mechanism, a substrate P binds twice to the enzyme, once in its physiological position and the second time in a nonphysiological position, so the complexes PE and PEP are formed. Note that complex EP is different from the complex PE. The full rate equation (Equation II) is obtained by adding the terms $P/K_X + P^2/\alpha K_X K_{iP}$ into the denominator of Equation (I). In Equation (II), two new dissociation constants appear, K_X and αK_X.

The second special case is the Rapid equilibrium ordered Bi Bi mechanism with QE and QEQ complexes.

Mechanism 11 (Scheme 3).



$$v_o = \frac{V_1\left(\frac{AB}{K_{iA}K_B}\right) - V_2\left(\frac{PQ}{K_P K_{iQ}}\right)}{1 + \frac{A}{K_{iA}} + \frac{Q}{K_{iQ}} + \frac{AB}{K_{iA}K_B} + \frac{PQ}{K_P K_{iQ}} + \frac{Q}{K_X} + \frac{Q^2}{\alpha K_X K_{iQ}}} \quad \text{(III)}$$

Scheme 3.

In this ordered mechanism, the complexes EB, EP, EAP, and EBQ do not form, but a substrate Q binds twice to the enzyme, once in its physiological position and the second time in a nonphysiological position, so the complexes QE and QEQ are formed. The full rate equation (Equation III) is obtained simply by adding the terms $Q/K_X + Q^2/\alpha K_X K_{iQ}$ into the denominator of Equation (I), and by omitting the terms for EB, EP, EAP, and EBQ.

Derivation of rate equations in the double reciprocal form

The purpose of this work was to derive the product inhibition equations from rate equations in the double reciprocal form, for major sequential bisubstrate mechanisms. The derivation of rate equations in the double reciprocal form is really simple. Take for example the Rapid equilibrium random mechanism with EAP and EBQ complexes (Mechanism 4 in Table I). One takes the inverse form of the full rate equation (Equation I) and calculates the inverse of the initial rate ($1/v_o$) for A in the presence of B and Q, for B in the presence of A and Q, for A in the presence of B and P, and so on; in doing so, the terms with the missing substrate are always deleted. Note that V_2 in Equation (I) is zero if only A, B, and Q are present, as well as if only A, B, and P are present; the same is true for V_1 in the reverse direction. Since there are two substrates and two products of reaction, we obtain four product inhibition equations in the forward and four in the reverse direction (Equations 25–32).

From the full rate equation, for each of 11 mechanisms, eight product inhibition equations were derived in the double reciprocal form, a total of $11 \times 8 = 88$ equations, and presented below (Equations 1–88).

Rapid equilibrium random Bi Bi without dead-end complexes

$$\text{A}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left(1+\frac{K_\text{B}}{B}\right) + \frac{K_\text{A}}{V_1}\left[1+\frac{K_\text{iB}}{B}\left(1+\frac{Q}{K_\text{iQ}}\right)\right]\frac{1}{A} \tag{1}$$

$$\text{B}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left(1+\frac{K_\text{A}}{A}\right) + \frac{K_\text{B}}{V_1}\left[1+\frac{K_\text{iA}}{A}\left(1+\frac{Q}{K_\text{iQ}}\right)\right]\frac{1}{B} \tag{2}$$

$$\text{C}: \quad v_\text{o} = \frac{V_1\left(\frac{AB}{K_\text{iA}K_\text{B}}\right) - V_2\left(\frac{PQ}{K_\text{P}K_\text{iQ}}\right)}{1+\frac{A}{K_\text{iA}}+\frac{B}{K_\text{iB}}+\frac{P}{K_\text{iP}}+\frac{Q}{K_\text{iQ}}+\frac{AB}{K_\text{iA}K_\text{B}}+\frac{PQ}{K_\text{P}K_\text{iQ}}+\frac{BQ}{K_\text{iB}K_\text{iiQ}}+\frac{AP}{K_\text{iA}K_\text{iiP}}+\frac{P}{K_\text{X}}+\frac{P^2}{\alpha K_\text{X}K_\text{iP}}} \tag{11}$$

$$\text{C}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left(1+\frac{K_\text{B}}{B}\right) + \frac{K_\text{A}}{V_1}\left[1+\frac{K_\text{iB}}{B}\left(1+\frac{P}{K_\text{iP}}\right)\right]\frac{1}{A} \tag{3}$$

$$\text{D}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left(1+\frac{K_\text{A}}{A}\right) + \frac{K_\text{B}}{V_1}\left[1+\frac{K_\text{iA}}{A}\left(1+\frac{P}{K_\text{iP}}\right)\right]\frac{1}{B} \tag{4}$$

$$\text{E}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1+\frac{K_\text{P}}{P}\right) + \frac{K_\text{Q}}{V_2}\left[1+\frac{K_\text{iP}}{P}\left(1+\frac{A}{K_\text{iA}}\right)\right]\frac{1}{Q} \tag{5}$$

$$\text{F}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1+\frac{K_\text{Q}}{Q}\right) + \frac{K_\text{P}}{V_2}\left[1+\frac{K_\text{iQ}}{Q}\left(1+\frac{A}{K_\text{iA}}\right)\right]\frac{1}{P} \tag{6}$$

$$\text{G}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1+\frac{K_\text{P}}{P}\right) + \frac{K_\text{Q}}{V_2}\left[1+\frac{K_\text{iP}}{P}\left(1+\frac{B}{K_\text{iB}}\right)\right]\frac{1}{Q} \tag{7}$$

$$\text{H}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1+\frac{K_\text{Q}}{Q}\right) + \frac{K_\text{P}}{V_2}\left[1+\frac{K_\text{iQ}}{Q}\left(1+\frac{B}{K_\text{iB}}\right)\right]\frac{1}{P} \tag{8}$$

Rapid equilibrium random Bi Bi with dead-end EAP complex

$$\text{A}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left(1+\frac{K_\text{B}}{B}\right) + \frac{K_\text{A}}{V_1}\left[1+\frac{K_\text{iB}}{B}\left(1+\frac{Q}{K_\text{iQ}}\right)\right]\frac{1}{A} \tag{9}$$

$$\text{B}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left(1+\frac{K_\text{A}}{A}\right) + \frac{K_\text{B}}{V_1}\left[1+\frac{K_\text{iA}}{A}\left(1+\frac{Q}{K_\text{iQ}}\right)\right]\frac{1}{B} \tag{10}$$

$$\text{D}: \quad \frac{1}{v_\text{o}} = \frac{1}{\text{V}_1}\left(1+\frac{K_\text{A}}{A}\right) + \frac{K_\text{B}}{V_1}\left[1+\frac{K_\text{iA}}{A}\left(1+\frac{P}{K_\text{iP}}\right)+\frac{P}{K_\text{iiP}}\right]\frac{1}{B} \tag{12}$$

$$\text{E}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1+\frac{K_\text{P}}{P}\right) + \frac{K_\text{Q}}{V_2}\left[1+\frac{K_\text{iP}}{P}\left(1+\frac{A}{K_\text{iA}}\right)+\frac{A}{K_\text{iiA}}\right]\frac{1}{Q} \tag{13}$$

$$\text{F}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left[1+\frac{K_\text{Q}}{Q}\left(1+\frac{A}{K_\text{iiA}}\right)\right] + \frac{K_\text{P}}{V_2}\left[1+\frac{K_\text{iQ}}{Q}\left(1+\frac{A}{K_\text{iA}}\right)\right]\frac{1}{P} \tag{14}$$

$$\text{G}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1+\frac{K_\text{P}}{P}\right) + \frac{K_\text{Q}}{V_2}\left[1+\frac{K_\text{iP}}{P}\left(1+\frac{B}{K_\text{iB}}\right)\right]\frac{1}{Q} \tag{15}$$

$$\text{H}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1+\frac{K_\text{Q}}{Q}\right) + \frac{K_\text{P}}{V_2}\left[1+\frac{K_\text{iQ}}{Q}\left(1+\frac{B}{K_\text{iB}}\right)\right]\frac{1}{P} \tag{16}$$

Rapid equilibrium random Bi Bi with dead-end EBQ complex

$$\text{A}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_B}{B}\right) + \frac{K_A}{V_1}\left[1 + \frac{K_{iB}}{B}\left(1 + \frac{Q}{K_{iQ}}\right) + \frac{Q}{K_{iiQ}}\right]\frac{1}{A} \quad (17)$$

$$\text{B}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left[1 + \frac{K_A}{A}\left(1 + \frac{Q}{K_{iiQ}}\right)\right] + \frac{K_B}{V_1}\left[1 + \frac{K_{iA}}{A}\left(1 + \frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \quad (18)$$

$$\text{C}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_B}{B}\right) + \frac{K_A}{V_1}\left[1 + \frac{K_{iB}}{B}\left(1 + \frac{P}{K_{iP}}\right)\right]\frac{1}{A} \quad (19)$$

$$\text{D}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_A}{A}\right) + \frac{K_B}{V_1}\left[1 + \frac{K_{iA}}{A}\left(1 + \frac{P}{K_{iP}}\right)\right]\frac{1}{B} \quad (20)$$

$$\text{E}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1 + \frac{K_P}{P}\right) + \frac{K_Q}{V_2}\left[1 + \frac{K_{iP}}{P}\left(1 + \frac{A}{K_{iA}}\right)\right]\frac{1}{Q} \quad (21)$$

$$\text{F}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1 + \frac{K_Q}{Q}\right) + \frac{K_P}{V_2}\left[1 + \frac{K_{iQ}}{Q}\left(1 + \frac{A}{K_{iA}}\right)\right]\frac{1}{P} \quad (22)$$

$$\text{G}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left[1 + \frac{K_P}{P}\left(1 + \frac{B}{K_{iiB}}\right)\right] + \frac{K_Q}{V_2}\left[1 + \frac{K_{iP}}{P}\left(1 + \frac{B}{K_{iB}}\right)\right]\frac{1}{Q} \quad (23)$$

$$\text{H}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1 + \frac{K_Q}{Q}\right) + \frac{K_P}{V_2}\left[1 + \frac{K_{iQ}}{Q}\left(1 + \frac{B}{K_{iB}}\right) + \frac{B}{K_{iiB}}\right]\frac{1}{P} \quad (24)$$

Rapid equilibrium random BiBi with dead-end EAP and EBQ complexes

$$\text{A}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_B}{B}\right) + \frac{K_A}{V_1}\left[1 + \frac{K_{iB}}{B}\left(1 + \frac{Q}{K_{iQ}}\right) + \frac{Q}{K_{iiQ}}\right]\frac{1}{A} \quad (25)$$

$$\text{B}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left[1 + \frac{K_A}{A}\left(1 + \frac{Q}{K_{iiQ}}\right)\right] + \frac{K_B}{V_1}\left[1 + \frac{K_{iA}}{A}\left(1 + \frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \quad (26)$$

$$\text{C}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left[1 + \frac{K_B}{B}\left(1 + \frac{P}{K_{iiP}}\right)\right] + \frac{K_A}{V_1}\left[1 + \frac{K_{iB}}{B}\left(1 + \frac{P}{K_{iP}}\right)\right]\frac{1}{A} \quad (27)$$

$$\text{D}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_A}{A}\right) + \frac{K_B}{V_1}\left[1 + \frac{K_{iA}}{A}\left(1 + \frac{P}{K_{iP}}\right) + \frac{P}{K_{iiP}}\right]\frac{1}{B} \quad (28)$$

$$\text{E}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1 + \frac{K_P}{P}\right) + \frac{K_Q}{V_2}\left[1 + \frac{K_{iP}}{P}\left(1 + \frac{A}{K_{iA}}\right) + \frac{A}{K_{iiA}}\right]\frac{1}{Q} \quad (29)$$

$$\text{F}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left[1 + \frac{K_Q}{Q}\left(1 + \frac{A}{K_{iiA}}\right)\right] + \frac{K_P}{V_2}\left[1 + \frac{K_{iQ}}{Q}\left(1 + \frac{A}{K_{iA}}\right)\right]\frac{1}{P} \quad (30)$$

$$\text{G}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left[1 + \frac{K_P}{P}\left(1 + \frac{B}{K_{iiB}}\right)\right] + \frac{K_Q}{V_2}\left[1 + \frac{K_{iP}}{P}\left(1 + \frac{B}{K_{iB}}\right)\right]\frac{1}{Q} \quad (31)$$

$$\text{H}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1 + \frac{K_Q}{Q}\right) + \frac{K_P}{V_2}\left[1 + \frac{K_{iQ}}{Q}\left(1 + \frac{B}{K_{iB}}\right) + \frac{B}{K_{iiB}}\right]\frac{1}{P} \quad (32)$$

Rapid equilibrium random Bi Bi with dead-end EAP, EBQ, PE, and PEP complexes

$$\text{A}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_B}{B}\right)+\frac{K_A}{V_1}\left[1+\frac{K_{iB}}{B}\left(1+\frac{Q}{K_{iQ}}\right)+\frac{Q}{K_{iiQ}}\right]\frac{1}{A} \tag{33}$$

$$\text{B}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left[1+\frac{K_A}{A}\left(1+\frac{Q}{K_{iiQ}}\right)\right]+\frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \tag{34}$$

$$\text{C}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left[1+\frac{K_B}{B}\left(1+\frac{P}{K_{iiP}}\right)\right]+\frac{K_A}{V_1}\times\left[1+\frac{K_{iB}}{B}\left(1+\frac{P}{K_{iP}}+\frac{P}{K_X}+\frac{P^2}{\alpha K_X K_{iP}}\right)\right]\frac{1}{A} \tag{35}$$

$$\text{D}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_A}{A}\right)+\frac{K_B}{V_1}\times\left[1+\frac{K_{iA}}{A}\left(1+\frac{P}{K_{iP}}+\frac{P}{K_X}+\frac{P^2}{\alpha K_X K_{iP}}\right)+\frac{P}{K_{iiP}}\right]\frac{1}{B} \tag{36}$$

$$\text{E}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\frac{K_Q}{V_2}\left[1+\frac{K_{iP}}{P}\left(1+\frac{A}{K_{iA}}+\frac{P}{K_x}+\frac{P^2}{\alpha K_x K_{iP}}\right)+\frac{A}{K_{iiA}}\right]\frac{1}{Q} \tag{37}$$

$$\text{F}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_Q}{Q}\left(1+\frac{A}{K_{iiA}}+\frac{K_{iP}}{K_x}\right)\right]+\frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}+\frac{P^2}{\alpha K_x K_{iP}}\right)\right]\frac{1}{P} \tag{38}$$

$$\text{G}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_P}{P}\left(1+\frac{B}{K_{iiB}}\right)\right]+\frac{K_Q}{V_2}\left[1+\frac{K_{iP}}{P}\left(1+\frac{B}{K_{iB}}+\frac{P^2}{\alpha K_x K_{iP}}\right)+\frac{K_{iP}}{K_x}\right]\frac{1}{Q} \tag{39}$$

$$\text{H}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_Q}{Q}\left(1+\frac{K_{iP}}{K_x}\right)\right]+\frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{B}{K_{iB}}+\frac{P^2}{\alpha K_x K_{iP}}\right)+\frac{B}{K_{iiB}}\right]\frac{1}{P} \tag{40}$$

Rapid equilibrium ordered Bi Bi without dead-end complexes

$$\text{A}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_B}{B}\right)+\frac{K_{iA}K_B}{V_1 B}\left(1+\frac{Q}{K_{iQ}}\right)\frac{1}{A} \tag{41}$$

$$\text{B}: \quad \frac{1}{v_o}=\frac{1}{V_1}+\frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \tag{42}$$

$$\text{C}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_B}{B}\right)+\left(\frac{K_{iA}K_B}{V_1 B}\right)\frac{1}{A} \tag{43}$$

$$\text{D}: \quad \frac{1}{v_o}=\frac{1}{V_1}+\frac{K_B}{V_1}\left(1+\frac{K_{iA}}{A}\right)\frac{1}{B} \tag{44}$$

$$\text{E}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\frac{K_{iQ}K_P}{V_2 P}\left(1+\frac{A}{K_{iA}}\right)\frac{1}{Q} \tag{45}$$

$$\text{F}: \quad \frac{1}{v_o}=\frac{1}{V_2}+\frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{P} \tag{46}$$

$$\text{G}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\left(\frac{K_{iQ}K_P}{V_2 P}\right)\frac{1}{Q} \tag{47}$$

$$\text{H}: \quad \frac{1}{v_o}=\frac{1}{V_2}+\frac{K_P}{V_2}\left(1+\frac{K_{iQ}}{Q}\right)\frac{1}{P} \tag{48}$$

Rapid equilibrium ordered Bi Bi with dead-end EAP complex.

$$\text{A}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_B}{B}\right)+\frac{K_{iA}}{V_1}\frac{K_B}{B}\left(1+\frac{Q}{K_{iQ}}\right)\frac{1}{A} \tag{49}$$

$$\text{B}: \quad \frac{1}{v_o}=\frac{1}{V_1}+\frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \tag{50}$$

$$\text{C}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left[1+\frac{K_B}{B}\left(1+\frac{P}{K_{iiP}}\right)\right]+\left(\frac{K_{iA}}{V_1}\frac{K_B}{B}\right)\frac{1}{A} \tag{51}$$

$$\text{D}: \quad \frac{1}{v_o}=\frac{1}{V_1}+\frac{K_B}{V_1}\left(1+\frac{K_{iA}}{A}+\frac{P}{K_{iiP}}\right)\frac{1}{B} \tag{52}$$

$$\text{E}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\frac{K_{iQ}}{V_2}\frac{K_P}{P}\times\left[1+\frac{A}{K_{iA}}\left(1+\frac{P}{K_{iiP}}\right)\right]\frac{1}{Q} \tag{53}$$

$$\text{F}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_{iQ}K_PA}{K_{iA}K_{iiP}Q}\right)+\frac{K_P}{V_2}\times\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{P} \tag{54}$$

$$\text{G}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\left(\frac{K_{iQ}}{V_2}\frac{K_P}{P}\right)\frac{1}{Q} \tag{55}$$

$$\text{H}: \quad \frac{1}{v_o}=\frac{1}{V_2}+\frac{K_P}{V_2}\left(1+\frac{K_{iQ}}{Q}\right)\frac{1}{P} \tag{56}$$

Rapid equilibrium ordered Bi Bi with dead-end EBQ complex

$$\text{A}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_B}{B}\right)+\frac{K_{iA}}{V_1}\frac{K_B}{B}\times\left[1+\frac{Q}{K_{iQ}}\left(1+\frac{B}{K_{iiB}}\right)\right]\frac{1}{A} \tag{57}$$

$$\text{B}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_{iA}K_BQ}{K_{iiB}K_{iQ}A}\right)+\frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \tag{58}$$

$$\text{C}: \quad \frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_B}{B}\right)+\left(\frac{K_{iA}}{V_1}\frac{K_B}{B}\right)\frac{1}{A} \tag{59}$$

$$\text{D}: \quad \frac{1}{v_o}=\frac{1}{V_1}+\frac{K_B}{V_1}\left(1+\frac{K_{iA}}{A}\right)\frac{1}{B} \tag{60}$$

$$\text{E}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\frac{K_{iQ}}{V_2}\frac{K_P}{P}\left(1+\frac{A}{K_{iA}}\right)\frac{1}{Q} \tag{61}$$

$$\text{F}: \quad \frac{1}{v_o}=\frac{1}{V_2}+\frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{P} \tag{62}$$

$$\text{G}: \quad \frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_P}{P}\left(1+\frac{B}{K_{iiB}}\right)\right]+\left(\frac{K_{iQ}}{V_2}\frac{K_P}{P}\right)\frac{1}{Q} \tag{63}$$

$$\text{H}: \quad \frac{1}{v_o}=\frac{1}{V_2}+\frac{K_P}{V_2}\left(1+\frac{K_{iQ}}{Q}+\frac{B}{K_{iiB}}\right)\frac{1}{P} \tag{64}$$

Forward rapid equilibrium random reverse rapid equilibrium ordered Bi Bi

$$\text{A}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1+\frac{K_B}{B}\right) + \frac{K_A}{V_1}\left[1+\frac{K_{iB}}{B}\left(1+\frac{Q}{K_{iQ}}\right)\right]\frac{1}{A} \tag{65}$$

$$\text{B}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1+\frac{K_A}{A}\right) + \frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \tag{66}$$

$$\text{C}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1+\frac{K_B}{B}\right) + \frac{K_A}{V_1}\left(1+\frac{K_{iB}}{B}\right)\frac{1}{A} \tag{67}$$

$$\text{D}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1+\frac{K_A}{A}\right) + \frac{K_B}{V_1}\left(1+\frac{K_{iA}}{A}\right)\frac{1}{B} \tag{68}$$

$$\text{E}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1+\frac{K_P}{P}\right) + \frac{K_{iQ}}{V_2}\frac{K_P}{P}\left(1+\frac{A}{K_{iA}}\right)\frac{1}{Q} \tag{69}$$

$$\text{F}: \quad \frac{1}{v_o} = \frac{1}{V_2} + \frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{P} \tag{70}$$

$$\text{G}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1+\frac{K_P}{P}\right) + \frac{K_{iQ}}{V_2}\frac{K_P}{P}\left(1+\frac{B}{K_{iB}}\right)\frac{1}{Q} \tag{71}$$

$$\text{H}: \quad \frac{1}{v_o} = \frac{1}{V_2} + \frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{B}{K_{iB}}\right)\right]\frac{1}{P} \tag{72}$$

Forward rapid equilibrium ordered reverse rapid equilibrium random Bi Bi

$$\text{A}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1+\frac{K_B}{B}\right) + \frac{K_{iA}}{V_1}\frac{K_B}{B}\left(1+\frac{Q}{K_{iQ}}\right)\frac{1}{A} \tag{73}$$

$$\text{B}: \quad \frac{1}{v_o} = \frac{1}{V_1} + \frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \tag{74}$$

$$\text{C}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1+\frac{K_B}{B}\right) + \frac{K_{iA}K_B}{V_1 B}\left(1+\frac{P}{K_{iP}}\right)\frac{1}{A} \tag{75}$$

$$\text{D}: \quad \frac{1}{v_o} = \frac{1}{V_1} + \frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{P}{K_{iP}}\right)\right]\frac{1}{B} \tag{76}$$

$$\text{E}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1+\frac{K_P}{P}\right) + \frac{K_Q}{V_2}\left[1+\frac{K_{iP}}{P}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{Q} \tag{77}$$

$$\text{F}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1+\frac{K_Q}{Q}\right) + \frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{P} \tag{78}$$

$$\text{G}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1+\frac{K_P}{P}\right) + \frac{K_Q}{V_2}\left(1+\frac{K_{iP}}{P}\right)\frac{1}{Q} \tag{79}$$

$$\text{H}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1+\frac{K_Q}{Q}\right) + \frac{K_P}{V_2}\left(1+\frac{K_{iQ}}{Q}\right)\frac{1}{P} \tag{80}$$

Rapid equilibrium ordered Bi Bi with dead-end QE and QEQ complex

$$\text{A}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_B}{B}\right) + \frac{K_{iA}}{V_1}\frac{K_B}{B} \times \left(1 + \frac{Q}{K_{iQ}} + \frac{Q}{K_X} + \frac{Q^2}{\alpha K_X K_{iQ}}\right)\frac{1}{A} \tag{81}$$

$$\text{B}: \quad \frac{1}{v_o} = \frac{1}{V_1} + \frac{K_B}{V_1} \times \left[1 + \frac{K_{iA}}{A}\left(1 + \frac{Q}{K_{iQ}} + \frac{Q}{K_X} + \frac{Q^2}{\alpha K_X K_{iQ}}\right)\right]\frac{1}{B} \tag{82}$$

$$\text{C}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_B}{B}\right) + \left(\frac{K_{iA}}{V_1}\frac{K_B}{B}\right)\frac{1}{A} \tag{83}$$

$$\text{D}: \quad \frac{1}{v_o} = \frac{1}{V_1} + \frac{K_B}{V_1}\left(1 + \frac{K_{iA}}{A}\right)\frac{1}{B} \tag{84}$$

$$\text{E}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left[1 + \frac{K_P}{P}\left(1 + \frac{K_{iQ}}{K_X}\right)\right] + \frac{K_{iQ}K_P}{V_2 P}\left(1 + \frac{A}{K_{iA}} + \frac{Q^2}{\alpha K_X K_{iQ}}\right)\frac{1}{Q} \tag{85}$$

$$\text{F}: \quad \frac{1}{v_o} = \frac{1}{V_2} + \frac{K_P}{V_2}\left[1 + \frac{K_{iQ}}{Q}\left(1 + \frac{A}{K_{iA}}\right) + \frac{K_{iQ}}{K_X} + \frac{Q}{\alpha K_X}\right]\frac{1}{P} \tag{86}$$

$$\text{G}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left[1 + \frac{K_P}{P}\left(1 + \frac{K_{iQ}}{K_X}\right)\right] + \frac{K_{iQ}K_P}{V_2 P}\left(1 + \frac{Q^2}{\alpha K_X K_{iQ}}\right)\frac{1}{Q} \tag{87}$$

$$\text{H}: \quad \frac{1}{v_o} = \frac{1}{V_2} + \frac{K_P}{V_2}\left(1 + \frac{K_{iQ}}{K_X} + \frac{K_{iQ}}{Q} + \frac{Q}{\alpha K_X}\right)\frac{1}{P} \tag{88}$$

Product inhibition patterns. From Equations (1–88), one can obtain 88 different product inhibition patterns, 44 in the forward and 44 in the reverse direction. Thus, the product inhibition patterns listed in Table II, are constructed from the product inhibition Equations (1–88). The primary plots, listed in Table II, show the effect of product inhibitors on intercepts in double-reciprocal plots. Note that for mechanisms 1–5, the patterns in the forward direction are symmetrical to patterns in the reverse direction; in mechanisms 6–11 this symmetry is absent. All double reciprocal plots (primary plots) are linear.

Table III shows the effect of inhibitors on slope replots; slope replots are obtained from the primary

Table II. Effect of product inhibitors on intercepts of primary plots in rapid equilibrium Bi Bi mechanisms.

		Product inhibitor				Product inhibitor			
		Q		P		A		B	
		Varied substrate				Varied substrate			
Mechanism		1/A	1/B	1/A	1/B	1/Q	1/P	1/Q	1/P
Experiment		*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
1	RE Random Bi Bi without dead-end complexes	C	C	C	C	C	C	C	C
2	RE Random Bi Bi with EAP complex	C	C	NC	C	C	NC	C	C
3	RE Random Bi Bi with EBQ complex	C	NC	C	C	C	C	NC	C
4	RE Random Bi Bi with EAP and EBQ complexes	C	NC	NC	C	C	NC	NC	C
5	RE Random Bi Bi with EAP, EBQ, PE, and PEP complexes	C	NC	NC	C	C	NC	NC	C
6	RE Ordered Bi Bi without dead-end complexes	C	C	♣	♣	C	C	♣	♣
7	RE Ordered Bi Bi with dead-end EAP complex	C	C	UC	C	C	NC	♣	♣
8	RE Ordered Bi Bi with dead-end EBQ complex	C	NC	♣	♣	C	C	UC	C
9	Forward RE random, reverse RE ordered Bi Bi	C	C	♣	♣	C	C	C	C
10	Forward RE ordered, reverse RE random Bi Bi	C	C	C	C	C	C	♣	♣
11	RE ordered Bi Bi, with QE and QEQ complex	C	C	♣	♣	C	C	♣	♣

RE = rapid equilbirium; C = competitive; NC = noncompetitive; UC = uncompetitive; ♣ = product inhibitor has no effect on intercept or slope.

Table III. Slope replots in product inhibition patterns in rapid equilibrium Bi Bi mechanisms.

		Product inhibitor				Product inhibitor			
		Q		P		A		B	
		Varied substrate				Varied substrate			
Mechanism		1/A	1/B	1/A	1/B	1/Q	1/P	1/Q	1/P
Experiment		*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
1	RE Random Bi Bi without dead-end complexes	L	L	L	L	L	L	L	L
2	RE Random Bi Bi with EAP complex	L	L	L	L	L	L	L	L
3	RE Random Bi Bi with EBQ complex	L	L	L	L	L	L	L	L
4	RE Random Bi Bi with EAP and EBQ complexes	L	L	L	L	L	L	L	L
5	RE Random Bi Bi with EAP, EBQ,PE, and PEP complexes	L	L	NL	NL	L	L	L	L
6	RE Ordered Bi Bi without dead-end complexes	L	L	L/N	L/N	L	L	L/N	L/N
7	RE Ordered Bi Bi with dead-end EAP complex	L	L	L/N	L	L	L	L/N	L/N
8	RE Ordered Bi Bi with dead-end EBQ complex	L	L	L/N	L/N	L	L	L/N	L
9	RE Forward random, RE reverse ordered Bi Bi	L	L	L/N	L/N	L	L	L	L
10	RE Forward ordered, RE reverse random Bi Bi	L	L	L	L	L	L	L/N	L/N
11	RE ordered Bi Bi, with QE and QEQ complex	NL	NL	L/N	L/N	L	L	L/N	L/N

SS = steady-state; RE = rapid equilibrium; L = linear; NL = nonlinear; L/N = slopes in primary plots are linear, but not responding to increasing concentrations of product inhibitor.

plots. The nonlinear slope replots in secondary plots are obtained only in two cases, when one of reactants binds twice to the enzyme, in a sequential fashion (Mechanisms 4 and 11 in Table I). Thus, combining the informations obtained from primary and secondary plots, one may unambiguously identify each mechanism in Table I and distinguish it clearly from all others.

Steady-state Bi Bi mechanisms

In this section, we shall consider seven steady-state or mixed rapid equilibrium and steady-state mechanisms: steady-state ordered, Theorell-Chance, steady-state ordered with a rapid equilibrium segment, steady-state ordered with dead-end EB complex, steady-state ordered with dead-end EP complex, forward rapid equilibrium random and reverse steady-state ordered, and the steady-state random.

The derivation of rate equations for steady-state mechanisms is a difficult task compared to rapid equilibrium mechanisms. For bisubstrate mechanisms with two substrates and two products of reaction, it is necessary to use the manual procedures, the King-Altman method [12] the method of [1], or other methods [14]. The derivations are lengthy and require some pratice for an efficient application. Let us review the rate equations for steady-state mechanisms.

Mechanism 12. Steady-state ordered (Scheme 4).

$$E \rightleftharpoons EA \rightleftharpoons EAB \rightleftharpoons EPQ \rightleftharpoons EQ \rightleftharpoons E$$

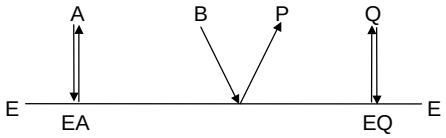
$$v_o = \frac{V_1 V_2 \left(AB - \frac{PQ}{K_{eq}} \right)}{V_2 K_{iA} K_B + V_2 K_B A + V_2 K_A B + \frac{V_1 K_Q}{K_{eq}} P + \frac{V_1 K_P}{K_{eq}} Q + V_2 AB + \frac{V_1 K_Q}{K_{iA} K_{eq}} AP + \frac{V_1}{K_{eq}} PQ + \frac{V_2 K_A}{K_{iQ}} BQ + \frac{V_2}{K_{iP}} ABP + \frac{V_1}{K_{iB} K_{eq}} BPQ} \quad \text{(IV)}$$

Scheme 4.

In this mechanism, the binding of substrates is ordered from both sides of reaction. The full rate equation is complex and contains more denominator terms then enzyme forms in the mechanism; contrary to that, the rapid equilibrium mechanisms always contain

one denominator term for each enzyme form in the mechanism.

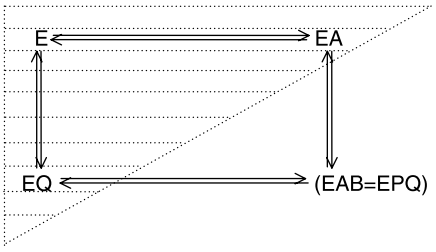
Mechanism 13. Theorell-Chance (Scheme 5).



Scheme 5.

The Theorell-Chance mechanism is a simplified version of an ordered mechanism where the steady-state level of central complexes is very low. Therefore, the rate equatioin is identical with the ordered mechanism, except that the terms in ABP and BPQ are missing from the denominator.

Mechanism 14. Steady-state ordered with rapid equilibrium segments (Scheme 6).



$$v_o = \frac{V_1 V_2 \left(AB - \dfrac{PQ}{K_{eq}} \right)}{V_2 K_{iA} K_B + V_2 K_B A + \dfrac{V_1 K_P}{K_{eq}} Q + V_2 AB + \dfrac{V_1}{K_{eq}} PQ} \quad \text{(V)}$$

Scheme 6.

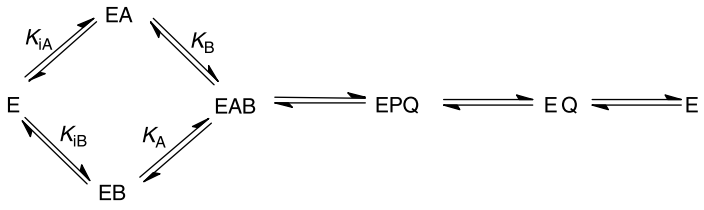
In *Scheme 6* the shaded area represents the rapid equilibrium segments. The rate equation (Equation V) has the same form as that for the rapid equilibrium ordered mechanism (Mechanism 6 in Table I), except that the kinetic constants associated with B and P are the Michaelis constants and the constants associated with A and Q are true dissociation constants.

Mechanism 15. Steady-state ordered with dead-end EB complex. In this ordered mechanism B binds to E to form an unproductive complex EB which cannot bind A. The rate equation has the same form as that for the steady-state ordered Bi Bi mechanism, except that denominator terms associated with $V_2 K_{iA} K_B$, B and P are multiplied with $(1 + B/K_X)$. A new constant K_X is the dissociation constant of B from the EB complex.

Mechanism 16. Steady-state ordered with dead-end EP complex. In this ordered mechanism P binds to E to form an unproductive complex EP which cannot bind Q. The rate equation again has the same form as that for the steady-state ordered Bi Bi mechanism, except that denominator terms associated with $V_2 K_{iA} K_B$, B and P are multiplied with $(1 + P/K_X)$. K_X is the dissociation constant of P from the EP complex.

Mechanism 17. Forward rapid equilibrium random, reverse steady-state ordered (Scheme 7). This mechanism was described by [10] in order to explain the kinetic mechanism of Asp49 mutant of yeast alcohol dehydrogenase.

Mechanism 18. Steady-state random Bi Bi mechanism. The last mechanism, a steady-state random Bi Bi, makes an exception to all other mechanisms. A full



$$v_o = \frac{V_1 V_2 \left(AB - \dfrac{PQ}{K_{eq}} \right)}{V_2 K_{iA} K_B + V_2 K_B A + V_2 K_A B + \dfrac{V_1 K_Q}{K_{eq}} P + \dfrac{V_1 K_P}{K_{eq}} Q + V_2 AB + \dfrac{V_1 K_Q}{K_{iA} K_{eq}} AP + \dfrac{V_1 K_Q}{K_{iB} K_{eq}} BP + \dfrac{V_1}{K_{eq}} PQ + \dfrac{V_2}{K_{iP}} ABP} \quad \text{(VI)}$$

Scheme 7.

rate equation for this mechanism contains four dozen of kinetic terms in the denominator and, therefore, it is completely unsuitable for practical work [14,17] consequently, the full rate equation is not presented.

Derivation of rate equations in the double-reciprocal form

The derivation of rate equations in the double-reciprocal form from the full rate equations is essentially the same as for the rapid equilibrium reactions. First, the terms in the denominator, which contain the product that is omitted, are deleted. Then, the remaining equation is inverted and sorted out into 8 product inhibition equations. Since we have 7 full rate equations (Mechanism 18 is not shown), a total of $7 \times 8 = 56$ product inhibition equations are presented below (Equations 89–136).

Steady-state ordered

$$\text{A}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_B}{B}\right) + \frac{K_A}{V_1}\left(1 + \frac{K_{iA}K_B}{K_A B}\right)\left(1 + \frac{Q}{K_{iQ}}\right)\frac{1}{A} \tag{89}$$

$$\text{B}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left[1 + \frac{K_A}{A}\left(1 + \frac{Q}{K_{iQ}}\right)\right] + \frac{K_B}{V_1}\left[1 + \frac{K_{iA}}{A}\left(1 + \frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \tag{90}$$

$$\text{C}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left[1 + \frac{K_B}{B}\left(1 + \frac{K_Q P}{K_{iQ}K_P}\right) + \frac{P}{K_{iP}}\right] + \frac{K_A}{V_1}\left[1 + \frac{K_{iA}}{K_A}\frac{K_B}{B}\left(1 + \frac{K_Q P}{K_{iQ}K_P}\right)\right]\frac{1}{A} \tag{91}$$

$$\text{D}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_A}{A} + \frac{P}{K_{iP}}\right) + \frac{K_B}{V_1}\left(1 + \frac{K_{iA}}{A}\right)\left(1 + \frac{K_Q P}{K_{iQ}K_P}\right)\frac{1}{B} \tag{92}$$

$$\text{E}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1 + \frac{K_P}{P}\right) + \frac{K_Q}{V_2}\left(1 + \frac{K_{iQ}K_P}{K_Q P}\right) \times \left(1 + \frac{A}{K_{iA}}\right)\frac{1}{Q} \tag{93}$$

$$\text{F}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left[1 + \frac{K_Q}{Q}\left(1 + \frac{A}{K_{iA}}\right)\right] + \frac{K_P}{V_2}\left[1 + \frac{K_{iQ}}{Q}\left(1 + \frac{A}{K_{iA}}\right)\right]\frac{1}{P} \tag{94}$$

$$\text{G}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left[1 + \frac{B}{K_{iB}} + \frac{K_P}{P}\left(1 + \frac{K_A B}{K_{iA}K_B}\right)\right] + \frac{K_Q}{V_2}\left[1 + \frac{K_{iQ}K_P}{K_Q P}\left(1 + \frac{K_A B}{K_{iA}K_B}\right)\right]\frac{1}{Q} \tag{95}$$

$$\text{H}: \quad \frac{1}{v_o} = \frac{1}{V_2}\left(1 + \frac{B}{K_{iB}} + \frac{K_Q}{Q}\right) + \frac{K_P}{V_2}\left(1 + \frac{K_{iQ}}{Q}\right)\left(1 + \frac{K_A B}{K_{iA}K_B}\right)\frac{1}{P} \tag{96}$$

Theorell-Chance

$$\text{A}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_B}{B}\right) + \frac{K_A}{V_1}\left(1 + \frac{K_{iB}}{B}\right)\left(1 + \frac{Q}{K_{iQ}}\right)\frac{1}{A} \tag{97}$$

$$B: \quad \frac{1}{v_o} = \frac{1}{V_1}\left[1 + \frac{K_A}{A}\left(1 + \frac{Q}{K_{iQ}}\right)\right] + \frac{K_B}{V_1}\left[1 + \frac{K_{iA}}{A}\left(1 + \frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \tag{98}$$

$$\text{C}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left[1 + \frac{K_B}{B}\left(1 + \frac{P}{K_{iP}}\right)\right] + \frac{K_A}{V_1}\left[1 + \frac{K_{iB}}{B}\left(1 + \frac{P}{K_{iP}}\right)\right]\frac{1}{A} \tag{99}$$

$$\text{D}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_A}{A}\right) + \frac{K_B}{V_1}\left(1 + \frac{K_{iA}}{A}\right) \times \left(1 + \frac{P}{K_{iP}}\right)\frac{1}{B} \tag{100}$$

E: $$\frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\frac{K_Q}{V_2}\left(1+\frac{K_{iP}}{P}\right)\left(1+\frac{A}{K_{iA}}\right)\frac{1}{Q} \quad (101)$$

F: $$\frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_Q}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]+\frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{P} \quad (102)$$

G: $$\frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_P}{P}\left(1+\frac{B}{K_{iB}}\right)\right]+\frac{K_Q}{V_2}\left[1+\frac{K_{iP}}{P}\left(1+\frac{B}{K_{iB}}\right)\right]\frac{1}{Q} \quad (103)$$

H: $$\frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_Q}{Q}\right)+\frac{K_P}{V_2}\left(1+\frac{K_{iQ}}{Q}\right)\left(1+\frac{B}{K_{iB}}\right)\frac{1}{P} \quad (104)$$

Steady-state ordered with a rapid equilibrium segment

A: $$\frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_B}{B}\right)+\frac{K_{iA}K_B}{V_1B}\left(1+\frac{Q}{K_{iQ}}\right)\frac{1}{A} \quad (105)$$

B: $$\frac{1}{v_o}=\frac{1}{V_1}+\frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{Q}{K_{iQ}}\right)\right]\frac{1}{B} \quad (106)$$

C: $$\frac{1}{v_o}=\frac{1}{V_1}\left(1+\frac{K_B}{B}\right)+\left(\frac{K_{iA}K_B}{V_1B}\right)\frac{1}{A} \quad (107)$$

D: $$\frac{1}{v_o}=\frac{1}{V_1}+\frac{K_B}{V_1}\left(1+\frac{K_{iA}}{A}\right)\frac{1}{B} \quad (108)$$

E: $$\frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\frac{K_{iQ}K_P}{V_2P}\left(1+\frac{A}{K_{iA}}\right)\frac{1}{Q} \quad (109)$$

F: $$\frac{1}{v_o}=\frac{1}{V_2}+\frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{P} \quad (110)$$

G: $$\frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\left(\frac{K_{iQ}K_P}{V_2P}\right)\frac{1}{Q} \quad (111)$$

H: $$\frac{1}{v_o}=\frac{1}{V_2}+\frac{K_P}{V_2}\left(1+\frac{K_{iQ}}{Q}\right)\frac{1}{P} \quad (112)$$

Steady-state ordered with a dead-end EB complex.

A: $$\frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_B}{B}\right)+\frac{K_A}{V_1}\left(1+\frac{K_{iA}K_B}{K_AB}\right)\left(1+\frac{Q}{K_{iQ}}+\frac{B}{K_X}\right)\frac{1}{A} \quad (113)$$

B: $$\frac{1}{v_o}=\frac{1}{V_1}\left[1+\frac{K_A}{A}\left(1+\frac{Q}{K_{iQ}}+\frac{K_{iA}K_B}{K_AK_X}\right)\right]+\frac{K_B}{V_1}\left[1+\frac{K_{iA}}{A}\left(1+\frac{Q}{K_{iQ}}\right)+\frac{K_AB^2}{K_BK_XA}\right]\frac{1}{B} \quad (114)$$

C: $$\frac{1}{v_o}=\frac{1}{V_1}\left[1+\frac{P}{K_{iP}}+\frac{K_B}{B}\left(1+\frac{K_QP}{K_{iQ}K_P}\right)\right]+\frac{K_A}{V_1}\times\left[\left(1+\frac{B}{K_X}\right)\left(1+\frac{K_{iA}K_B}{K_AB}\left(1+\frac{K_QP}{K_{iQ}K_P}\right)\right)\right]\frac{1}{A} \quad (115)$$

D: $$\frac{1}{v_o}=\frac{1}{V_1}\left[1+\frac{P}{K_{iP}}+\frac{K_A}{A}\times\left(1+\frac{K_{iA}K_B}{K_AK_X}\left(1+\frac{K_QP}{K_{iQ}K_P}\right)\right)\right]+\frac{K_B}{V_1}\left[\left(1+\frac{K_QP}{K_{iQ}K_P}\right)\left(1+\frac{K_{iA}}{A}\right)+\frac{K_AB^2}{K_BK_XA}\right]\frac{1}{B} \quad (116)$$

E: $$\frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\frac{K_Q}{V_2}\left(1+\frac{K_{iQ}K_P}{K_QP}\right)\left(1+\frac{A}{K_{iA}}\right)\frac{1}{Q} \quad (117)$$

F: $$\frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_Q}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]+\frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{A}{K_{iA}}\right)\right]\frac{1}{P} \quad (118)$$

G: $$\frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_P}{P}\left(1+\frac{K_AB}{K_{iA}K_B}\right)+\frac{B}{K_{iB}}\right]+\frac{K_Q}{V_2}\left[\left(1+\frac{K_{iQ}}{K_Q}\frac{K_P}{P}\left(1+\frac{K_AB}{K_{iA}K_B}\right)\right)\right]\times\left(1+\frac{B}{K_X}\right)\left]\frac{1}{Q}\right. \quad (119)$$

$$\text{H}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left[1 + \frac{K_\text{Q}}{Q}\left(1 + \frac{B}{K_\text{X}}\right) + \frac{B}{K_\text{iB}}\right] + \frac{K_\text{P}}{V_2}\left[1 + \frac{K_\text{A}B}{K_\text{iA}K_\text{B}} + \frac{K_\text{iQ}}{Q}\left(1 + \frac{K_\text{A}B}{K_\text{iA}K_\text{B}}\right) \times \left(1 + \frac{B}{K_\text{X}}\right)\right]\frac{1}{P} \tag{120}$$

Steady-state ordered Bi Bi with a dead-end Ep complex

$$\text{A}: \quad \frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_\text{B}}{\text{B}}\right) + \frac{K_A}{V_1}\left(1 + \frac{K_\text{iA}K_\text{B}}{\text{K}_\text{A}B}\right) \times \left(1 + \frac{Q}{\text{K}_\text{iQ}}\right)\frac{1}{A} \tag{121}$$

$$\text{B}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left[1 + \frac{K_\text{A}}{A}\left(1 + \frac{Q}{K_\text{iQ}}\right)\right] + \frac{K_\text{B}}{V_1}\left[1 + \frac{K_\text{iA}}{A}\left(1 + \frac{Q}{K_\text{iQ}}\right)\right]\frac{1}{B} \tag{122}$$

$$\text{C}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left[1 + \frac{K_\text{B}}{\text{B}}\left(1 + \frac{K_\text{Q}P}{K_\text{iQ}K_\text{P}}\right) + \frac{P}{K_\text{iP}}\right] + \frac{K_\text{A}}{V_1}\left[\left(1 + \frac{K_\text{iA}}{K_\text{A}}\frac{K_\text{B}}{B}\left(1 + \frac{K_\text{Q}P}{K_\text{iQ}K_\text{P}}\right)\right) \times \left(1 + \frac{P}{K_\text{X}}\right)\right]\frac{1}{A} \tag{123}$$

$$\text{D}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left[1 + \frac{K_\text{A}}{\text{A}}\left(1 + \frac{P}{K_\text{X}}\right) + \frac{P}{K_\text{iP}}\right] + \frac{K_\text{B}}{V_1}\left[1 + \frac{K_\text{Q}P}{K_\text{iQ}K_\text{P}} + \frac{K_\text{iA}}{\text{A}}\left(1 + \frac{K_\text{Q}P}{K_\text{iQ}K_\text{p}}\right) \times \left(1 + \frac{P}{K_\text{X}}\right)\right]\frac{1}{B} \tag{124}$$

$$\text{E}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1 + \frac{K_\text{P}}{\text{P}}\right) + \frac{K_\text{Q}}{V_2}\left(1 + \frac{K_\text{iQ}K_\text{P}}{K_\text{Q}P}\right) \times \left(1 + \frac{A}{K_\text{iA}} + \frac{P}{K_\text{X}}\right)\frac{1}{Q} \tag{125}$$

$$\text{F}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left[1 + \frac{K_\text{Q}}{Q}\left(1 + \frac{A}{K_\text{iA}} + \frac{K_\text{iQ}K_P}{K_\text{Q}K_\text{X}}\right)\right] + \frac{K_\text{P}}{V_2}\left[1 + \frac{K_\text{iQ}}{Q}\left(1 + \frac{A}{K_\text{iA}}\right) + \frac{K_\text{Q}P^2}{K_\text{P}K_\text{X}Q}\right]\frac{1}{P} \tag{126}$$

$$\text{G}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left[1 + \frac{B}{K_\text{iB}} + \frac{K_\text{P}}{P}\left(1 + \frac{K_\text{A}B}{K_\text{iA}K_\text{B}}\right)\right] + \frac{K_Q}{V_2}\left[\left(1 + \frac{P}{K_\text{X}}\right)\left(1 + \frac{K_\text{iQ}K_\text{P}}{K_\text{Q}P}\left(1 + \frac{K_\text{A}B}{K_\text{iA}K_\text{B}}\right)\right)\right]\frac{1}{Q} \tag{127}$$

$$\text{H}: \frac{1}{v_\text{o}} = \frac{1}{V_2}\left[1 + \frac{B}{K_\text{iB}} + \frac{K_\text{Q}}{Q}\left(1 + \frac{K_\text{iQ}K_\text{p}}{K_\text{Q}K_\text{X}}\left(1 + \frac{K_\text{A}B}{K_\text{iA}K_\text{B}}\right)\right)\right] + \frac{K_\text{P}}{V_2}\left[\left(1 + \frac{K_\text{A}B}{K_\text{iA}K_\text{B}}\right)\left(1 + \frac{K_\text{iQ}}{Q}\right) + \frac{K_\text{Q}P^2}{K_\text{P}K_\text{X}Q}\right]\frac{1}{P} \tag{128}$$

Forward rapid equilibrium random, reverse steady-state ordered

$$\text{A}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left(1 + \frac{K_\text{B}}{\text{B}}\right) + \frac{K_\text{A}}{V_1}\left[1 + \frac{K_\text{iB}}{B}\left(1 + \frac{Q}{K_\text{iQ}}\right)\right]\frac{1}{A} \tag{129}$$

$$\text{B}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left(1 + \frac{K_\text{A}}{A}\right) + \frac{K_\text{B}}{V_1}\left[1 + \frac{K_\text{iA}}{A}\left(1 + \frac{Q}{K_\text{iQ}}\right)\right]\frac{1}{B} \tag{130}$$

$$\text{C}: \frac{1}{v_\text{o}} = \frac{1}{V_1}\left[1 + \frac{K_\text{B}}{\text{B}}\left(1 + \frac{K_\text{Q}P}{K_\text{iQ}K_\text{P}}\right) + \frac{P}{K_\text{iP}}\right] + \frac{K_\text{A}}{V_1} \times \left[1 + \frac{K_\text{iB}}{B}\left(1 + \frac{K_\text{Q}P}{K_\text{iQ}K_\text{P}}\left(1 + \frac{B}{K_\text{iB}}\right)\right)\right]\frac{1}{A} \tag{131}$$

$$\text{D}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_1}\left[1 + \frac{K_\text{A}}{\text{A}}\left(1 + \frac{K_\text{Q}P}{K_\text{iQ}K_\text{P}}\right) + \frac{P}{K_\text{iP}}\right] + \frac{K_\text{B}}{V_1}\left(1 + \frac{K_\text{Q}P}{K_\text{iQ}K_\text{P}}\right)\left(1 + \frac{K_\text{iA}}{A}\right)\frac{1}{B} \tag{132}$$

$$\text{E}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left(1 + \frac{K_\text{P}}{\text{P}}\right) + \frac{K_\text{Q}}{V_2} \times \left[1 + \frac{A}{K_\text{iA}} + \frac{K_\text{iQ}K_\text{P}}{K_\text{Q}P}\left(1 + \frac{A}{K_\text{iA}}\right)\right]\frac{1}{Q} \tag{133}$$

$$\text{F}: \quad \frac{1}{v_\text{o}} = \frac{1}{V_2}\left[1 + \frac{K_\text{Q}}{Q}\left(1 + \frac{A}{K_\text{iA}}\right)\right] + \frac{K_\text{P}}{V_2}\left[1 + \frac{K_\text{iQ}}{Q}\left(1 + \frac{A}{K_\text{iA}}\right)\right]\frac{1}{P} \tag{134}$$

Table IV. Effect of product inhibitors on intercepts of primary plots in steady-state Bi Bi mechanisms.

		Product inhibitor				Product inhibitor			
		Q		P		A		B	
		Varied substrate				Varied substrate			
Mechanism		1/A	1/B	1/A	1/B	1/Q	1/P	1/Q	1/P
Experiment		*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
12	SS Ordered Bi Bi	C	NC	NC	NC	C	NC	NC	NC
13	SS Theorell-Chance	C	NC	NC	C	C	NC	NC	C
14	SS Ordered Bi Bi with RE segment	C	C	♣	♣	C	C	♣	♣
15	SS Ordered Bi Bi with dead-end EB complex	C	NC	NC	NC	C	NC	NC	NC
16	SS Ordered Bi Bi with dead-end EP complex	C	NC	NC	NC	C	NC	NC	NC
17	Forward RE random, reverse SS ordered Bi Bi	C	C	NC	NC	C	NC	C	NC
18	SS Random Bi Bi[a)]	NC	NC	NC	NC	NC	NC	NC	NC

SS = steady-state; RE = rapid equilbirium; C = competitive; NC = noncompetitive; ♣ = product inhibitor has no effect on intercept or slope.
[a)] All patterns are noncompetitive [Segel, 1975].

$$\text{G:}\quad \frac{1}{v_o}=\frac{1}{V_2}\left(1+\frac{K_P}{P}\right)+\frac{K_Q}{V_2}\left[1+\frac{B}{K_{iB}}+\frac{K_{iQ}K_P}{K_Q P}\left(1+\frac{B}{K_{iB}}\right)\right]\frac{1}{Q} \quad (135)$$

$$\text{H:}\quad \frac{1}{v_o}=\frac{1}{V_2}\left[1+\frac{K_Q}{Q}\left(1+\frac{B}{K_{iB}}\right)\right]+\frac{K_P}{V_2}\left[1+\frac{K_{iQ}}{Q}\left(1+\frac{B}{K_{iB}}\right)\right]\frac{1}{P} \quad (136)$$

Product inhibition patterns. The construction of product inhibition patterns from product inhibition equations is the same as in the case of rapid equilibrium mechanisms. Table IV shows the effect of product inhibitors on primary plots (intercept effects), and Table V shows the effect of inhibitors on secondary plots (slope effects).

Finally, we can conclude that, combining the information obtained from primary and secondary plots in Tables II – V, one may unambiguously identify each of the 18 bisubstrate mechanisms and distinguish it clearly from all others.

Practical examples

In this section, we shall illustrate the practical application of product inhibition studies, outlined above, for solving the kinetic mechanism of a specific enzyme. In doing so, we have chosen different preparations of yeast alcohol dehydrogenase from baker's yeast operating with different substrates. For the purpose of this study, three examples were

Table V. Slope replots in product inhibition patterns in steady-state Bi Bi mechanisms.

		Product inhibitor				Product inhibitor			
		Q		P		A		B	
		Varied substrate				Varied substrate			
Mechanism		1/A	1/B	1/A	1/B	1/Q	1/P	1/Q	1/P
Experiment		*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
12	SS Ordered Bi Bi	L	L	L	L	L	L	L	L
13	SS Theorell-Chance	L	L	L	L	L	L	L	L
14	SS Ordered Bi Bi with RE segment	L	L	L/N	L/N	L	L	L/N	NL
15	SS Ordered Bi Bi with dead-end EB complex	L	L	L	L	L	L	NL	NL
16	SS Ordered Bi Bi with dead-end EP complex	L	L	NL	NL	L	L	L	L
17	Forward RE random, reverse SS ordered Bi Bi	L	L	L	L	L	L	L	L
18	SS Random Bi Bi[a)]	NL	NL	NL	NL	NL	NL	NL	NL

SS = steady-state; RE = rapid equilbirium; L = linear; NL = nonlinear; L/N = slopes in primary plots are linear, but not responding to increasing concentrations of product inhibitor.
[a)] All patterns are nonlinear, but the non-linearity is difficult to detect.

Table VI. Product inhibition patterns in primary plots in practical examples.

	Substrate			Example 1	Example 2	Example 3
Experiment	Variable	Fixed	Product inhibitor	Wild type Ethanol/acetaldehyde[a)] [9]	Asn49 mutant Ethanol/acetaldehyde[a)] [10]	Wild type propan-2-ol/ acetone[b)] [18]
A	A	B	Q	C	C	C
B	B	A	Q		C	NC
C	A	B	P		NC	NC
D	B	A	P	NC		C
E	Q	P	A	C	C	C
F	P	Q	A		NC	NC
G	Q	P	B		NC	NC
H	P	Q	B	NC		C

C = competitive; NC = noncompetitive.
[a)] At pH 7.3;[b)] At pH 7.0.

examined which are dealing extensively and in detail with kinetic studies of yeast alcohol dehydrogenase reactions (Table VI).

Table VI shows the product inhibition patterns for three examples of the yeast enzyme-catalyzed reactions from the literature: the oxidation of ethanol by NAD^+ and the wild type yeast alcohol dehydrogenase ([9]), the oxidation of ethanol by NAD^+ and the Asn49 mutant of the same enzyme [10], and the oxidation of propan-2-ol by NAD^+ and the wild type yeast alcohol dehydrogenase [18].

The assignment of substrates in Table VI was the same as outlined in the above sections: A was assigned to oxidized coenzyme and B to alcohol substrate in the forward direction, P was assigned to carbonyl substrate and Q to reduced coenzyme in the reverse direction.

Example 1

Oxidation of ethanol by NAD^+ and the wild type yeast alcohol dehydrogenase [9]. The product inhibition patterns in primary plots in this case are compatible with the steady-state ordered Bi Bi mechanism (Mechanism 12); acetaldehyde could be shown to be noncompetitive against ethanol only in very precise experiments [19]. The kinetic isotope effects in the forward direction were ${}^{D}V_1 = 1.8$, ${}^{D}V_1/K_A = 1.8$, and ${}^{D}V_1/K_B = 3.2$, compatible with the ordered addition of substrates [10]. In the reverse direction, the kinetic isotope effects were close to unity, ${}^{D}V_2 = 0.7$, ${}^{D}V_2/K_Q = 0.9$, and ${}^{D}V_2/K_P = 1.3$, suggesting the absence of isotope-sensitive steps in this direction [10]. The still significant value of ${}^{D}V_1/K_A$ is probably due to dissociation of NAD^+ from the ternary complex, as suggested by [7].

A further kinetic complication is a double-reciprocal plot for inhibition of acetaldehyde by ethanol, which is noncompetitive, but the lines have no common intersection point to the left of the vertical axis as was shown by [8]; this indicates the presence of an extra complex in the mechanism. In summary, the kinetic isotope effects are compatible with the steady-state ordered mechanism overall, with some dissociation of reduced coenzyme from the ternary complex.

Example 2

Oxidation of ethanol by NAD^+ and Asn49 mutant of yeast alcohol dehydrogenase ([10]). The product inhibition patterns in primary plots in this case are compatible with the forward rapid equilibrium random and reverse steady-state ordered Bi Bi mechanism (Mechanism 17).

One kinetic complication, reported by [10], is the observation of a noncompetitive double-reciprocal plot for inhibition of NADH by ethanol (entry *G* in Table VI), which however, should be competitive according to Equation (135). The primary kinetic isotope effects in the forward direction, ${}^{D}V_1 = 2.1$, ${}^{D}V_1/K_A = 2.2$, and ${}^{D}V_1/K_B = 2.3$ were compatible with the random addition of substrates. In the reverse direction the kinetic isotope effects were not significantly different from the wild type enzyme, ${}^{D}V_2 = 1.2$, ${}^{D}V_2/K_Q = 1.3$, and ${}^{D}V_2/K_P = 1.4$, suggesting high commitment factors in the reverse direction; these results are not consistent with the rapid equilibrium assumption. In summary, it appears that Mechanism 17 describes the simplest case compatible with experimental data.

Example 3

Oxidation of propan-2-ol by NAD^+ and wild type yeast alcohol dehydrogenase [15,16,18]. The product inhibition patterns in primary plots, for this case, are in agreement with mechanisms 4, 5 and 13 (Table I, *Scheme 5*). Since the slope replot for experiment *D* in Table IV was nonlinear (Figure 1), it was concluded that Mechanism 5 in Table I, a

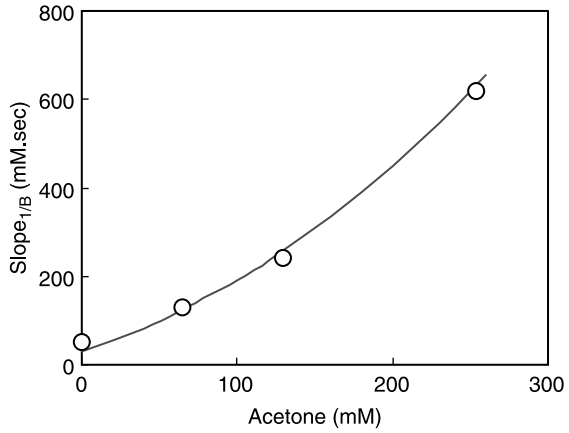


Figure 1. Fit of the slope function from Equation (VII) (————) to experimentally determined slope function (o) for inhibition of propan-2-ol with acetone, at constant NAD^+, which were measured in experiment [18].

rapid equilibrium random mechanism with two additional dead-end complexes, PE and PEP, was in question.

A further kinetic complication in this case was the observation of a linear slope replot in experiment *C* in Table VI (data not shown), which however, should be nonlinear according to the Mechanism 5 in Table IV; this corresponds to Equation (35). Therefore, the Mechanism 5 in Table I must be regarded only as the closest description of experimental data.

Further kinetic analysis was performed by fitting the slope function in Equation (VI) for variable B (propan-2-ol) in the presence of constant A (NAD^+) and increasing concentrations of P (acetone), to the slope data from the experiment.

$$\frac{1}{v_o} = \frac{1}{V_1}\left(1 + \frac{K_A}{A}\right) + \frac{K_B}{V_1} \times \left[1 + \frac{K_{iA}}{A}\left(1 + \frac{P}{K_{iP}} + \frac{P}{K_X} + \frac{P^2}{\alpha K_X K_{iP}}\right) + \frac{P}{K_{iiP}}\right]\frac{1}{B} \qquad \text{(VII)}$$

Figure 1 shows the fit of Equation (VII) to experimental data; Equation (VII) is identical to Equation (36). The fixed kinetic constants in this equation, $V_1 = 7\ s^{-1}$, $K_{iA} = 0.38$ mM, $K_{iB} = 117$ mM, $K_P = 477$ mM, $K_{iP} = 194$ mM, and $K_{iiP} = 170$ mM, were calculated from the data of [18] and inserted back into the equation. After this, the constants K_X and α were obtained from the best fit.

From the Figure 1 one can see that the combination of $K_X = 13.5$ mM and $\alpha = 1$, gives the best fit. However, other pairs of constants (such as $K_X = 10$ mM;$\alpha = 2$) provide a good fit as well which indicates that the two constants cannot be determined independently.

Conclusions

Product inhibition experiments are often the best means of distinguishing different mechanisms. However, they have their limitations which are illustrated above, in the practical section, with examples from the yeast alcohol dehydrogenase kinetics. The main limitations of the product inhibition diagnostics are as follows.

First, it is usually very difficult to detect the non-linearity of the primary plots if it is present. Second, it is easy to overlook the multiple intersection points in primary plots left to the vertical axis, if they are present. Third, it is sometimes difficult to detect a difference between the competitive and noncompetitive product inhibition pattern in primary plots, especially if the Michaelis and inhibition constant for the substrate are widely different. Fourth, it may be sometimes difficult to detect the non-linearity in slope or intercept replots in secondary plots. A failure to observe any of these anomalies will lead to erroneous conclusions about the mechanism involved.

There are two main sources of error in arriving at a correct mechanism from experimental data. First, one may easily overlook the existence of one (or more) enzyme-substrate complexes which were omitted from the mechanism. On the other hand, all enzyme forms may be accounted for in the mechanism, but the rapid equilibrium mechanism may have a steady-state segment and *vice versa*, a steady-state mechanism may have a rapid equilibrium segment. In both cases, the full rate equation for a real mechanism may become more complex and sometimes unmanageable for practical purposes.

For this reason, the analysis of a given kinetic mechanism, obtained with product inhibition studies, must be always expanded with other methods, preferably by the use of dead-end inhibitors and especially by the application of kinetic isotope effects [5,14].

The product inhibition patterns shown in Tables II–V have appeared to the extend of approximately one third in textbook format in Segel (1974) and in [14] books. The product inhibition equations (Equations 1–136) have appeared by approximately one fifth in the above textbooks. Therefore, this article is by far the most complete survey of product inhibition patterns and equations published so far.

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